Sexually Dimorphic Developmental Rates in the Caribbean Fruit Fly (Diptera: Tephritidae)

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ABSTRACT Female Caribbean fruit flies, *Anastrepha suspensa* (Loew), eclosed before males in field-collected and laboratory-reared samples. This dimorphism is due to more rapid female development during pupal, larval, and perhaps even egg stages. Adult females in the laboratory stock and in the field-collected samples eclosed before male members of the same cohort. At the conclusion of pupal development, 92% of females and only 52% of males emerged from the pupal state on the first of two days of eclosion. During the early portion of the larval maturation period, sex ratios were biased toward females and biased toward males at the end ($r^2 = 0.73$). Within 72 h of oviposition, 70% of female flies and only 55% of male flies had hatched. Earlier adult female eclosion does not necessarily result in earlier sexual maturation. Males copulated a day earlier than females ($\bar{x} = 6.6$ versus $\bar{x} = 7.5$ d of age). Earlier female larval development provides an opportunity to separate late-instar male and female maggots. At this point, larvae are still susceptible to attack by parasitic Hymenoptera (e.g., *Diachasmimorpha longicaudata* (Ashmead)). Mass-rearing facilities might thus be able to use female maggots to rear parasites for inundative releases while holding males for sterile release.

KEY WORDS Insecta, *Anastrepha suspensa*, Tephritidae, development

IN MANY INSECTS, males complete development before female siblings. This more rapid male maturation (protandry) can be sexually selected (Thornhill & Alcock 1983). In species with synchronized emergence, early males have the potentially greatest number of sexual opportunities (e.g., Gwynne 1980). In less synchronous species, earlier male development may reflect dimorphic nutritional needs and growth patterns. For example, the development time of the relatively enormous phengodid beetle female exceeds that of her male siblings by probably a year or more (Cicero 1988).

Instances of the opposite, female-first schedule (protogyny) are comparatively rare and have been described only in some odonates (Odonata: Aeshnidae), a few megachilid bees (Hymenoptera: Megachilidae), and a minority of the mosquitoes (Diptera: Culicidae) (Thornhill & Alcock 1983). Among tephritids, protogyny occurs in *Rhagoletis completa* Cresson (Diptera: Tephritidae) (Boyce 1934) and is suspected in *Phytalmia mouldsi* MacAlpine (Diptera: Tephritidae) (G. Dodson, personal communication). Earlier female eclosion also has been casually observed in the Caribbean fruit fly, *Anastrepha suspensa* (Loew), by insect rearers (J.M.S. & C.O.C., unpublished data). The major objective of our experiments was to examine sexual dimorphism in the developmental rate of *A. suspensa*, an important pest of subtropical fruit. Additionally, we were interested in the timing of sexual maturation and its relationship to sexual selection and developmental requirements. Dimorphic development and the timing of sexual maturation have implications for the mass rearing of these insects, either for sterile release or as hosts for inundatively released parasites.

Materials and Methods

Unless otherwise noted, Caribbean fruit flies were obtained from a colony maintained for >10 yr at the USDA–ARS Insect Attractants, Behavior, and Basic Biology Laboratory in Gainesville, Fla. Voucher specimens in the author's (J.M.S.) collection were deposited in the Florida State Collection of Arthropods, Division of Plant Industry, Gainesville, Fla.

Maggots were raised in the laboratory in the following manner. Eggs were collected daily from beeswax-covered cloth backs of screen cages (30 by 30 by 30 cm) containing several hundred adult flies. Eggs (≈16,000–20,000) were held on damp filter paper for 48 h and then placed on the surface of a diet material filling plastic trays (30 by 60 by 4 cm). Diet consisted of 62% water, 25% corn cob grits, 5% sugar, 4% torula yeast, 3% wheat germ, 1% hydrochloric acid, and trace amounts of P-methyl-hydrobenzoate and sodium benzoate to retard fungi. The diet was held for 4 d at 27°C, remoistened, and then held for another 4–5 d at 25°C. At this time, fully developed larvae began to surface, wander, and leave the diet. The trays were moistened once again and placed on blocks over damp vermiculite contained in a larger tray (38 by 76 by 4 cm). Mature maggots emerged from the diet, wandered about its surface, and eventually

fell into the vermiculite where they pupated within hours.

Sexual Dimorphism in Adult Eclosion. The search for sexual dimorphism in the development of *A. suspensa* focused on several easily defined intervals in the life cycle: egg hatch, larval maturation, duration of pupal stage, adult eclosion, and adult sexual maturation. To compare the timing of male and female eclosion in wild flies, four samples of fruit were collected in Dade County, Fla., during 1987–1988. The following types of fruit were gathered: in October, 52 guava (*Psidium guava* L.); in November, 52 guava and 18 Surinam cherries (*Eugenia longifolia* L.); in January, 8 guava, 122 loquat (*Eriobotrya japonica* (Thumb)), and 79 tropical almond (*Terminalia catappa* L.); and in April, 52 guava and 99 Surinam cherry. The fruit was held individually at 25°C and 80% RH in 450-ml plastic cups half filled with damp vermiculite. After 7 d, the vermiculite was sifted through a screen and *A. suspensa* pupae were counted and replaced. Pupae were held until eclosion and the number and sexes of flies noted. Statistical analysis was by χ^2 test (Zar 1974).

To substantiate findings from wild flies, mature maggots reared in the laboratory from a 24-h-long collection of eggs were held in damp vermiculite at 25°C and 80% RH (see rearing details in section on larval maturation). When eclosion began, flies were counted daily and separated by sex. Statistical analysis was by χ^2 test (Zar 1974).

Sexual Dimorphism in Duration of Pupal Period. Late-instar Caribbean fruit fly larvae were taken as they left laboratory diet trays prior to pupation. The resulting pupae were held individually in 35-ml fine-cloth-covered plastic cups half filled with damp vermiculite and maintained at 25°C and 80% RH until eclosion. Sex and length of the pupal stages were then determined. Statistical analysis was by χ^2 test (Zar 1974).

Sexual Dimorphism in Larval Maturation. Trays were first monitored for mature larvae leaving the diet to pupate on days 8 or 9 of larval development. When maggots first appeared, 50–100 active larvae were taken from the vermiculite tray by selecting those against one randomly chosen wall of the tray. If <50 were collected at that location, the remainder were taken from interior portions of the tray. Larvae were sampled at 6-h intervals (0600, 1200, 1800, 2400 hours) until the trays were discarded (3 or 4 d later). Larvae from a particular sample were placed on damp vermiculite in a 450-ml plastic cup and held at 25°C and 80% RH until eclosion. Adult flies were removed from cups and sexed on a daily basis until no further emergence occurred. Six replicate trays were sampled. The relationship between sample sex ratio and time of maturation was analyzed by correlation (SAS Institute 1982). Results from this experiment were substantiated by removing 100 larvae from similar trays on days 1 and 3 of larval wandering. These larvae were kept under the conditions described above and the sex ratio of the emerging adults noted. There were five replicates and means were compared by *t* test (SAS Institute 1982).

To determine what proportion of a larval cohort matures on any particular day, trays, as previously described, were examined at 6-h intervals (0600, 1200, and 1800 hours) over 4 d. Previous experience indicated that relatively few larvae leave the diet at 2400 hours so this sampling time was excluded. The number of mature maggots moving across the diet surface was counted for each sample. Ten trays were examined.

Mature larval weight (to 0.1 mg) was obtained on a balance (Model A30, Mettler, Hightown, N.J.). Weighed larvae were held individually in 35-ml cups half filled with damp vermiculite. The resulting adults were sexed. It could then be determined if mean larval weight differed between the sexes. Data were analyzed by *t* test (SAS Institute 1982).

Sexual Dimorphism in Egg Hatch. Eggs were obtained from the wax-impregnated-cloth backings of the previously described oviposition cages. The backings had been previously frozen to destroy any eggs they might carry. Backings were exposed to ovipositing females for 1 h and eggs were washed onto a piece of damp, black filter paper. The paper containing eggs was then placed in a closed plastic container, the bottom of which was covered with a film of water. The eggs were maintained at 25°C for 72 h. The paper was examined with a microscope for neonate larvae (previous experience suggested that the bulk of hatch occurs from 2.5–4 d after oviposition). These larvae were removed with a brush and placed on about 150 g of the previously described larval diet. The process was repeated 24 h later and the hatchlings put in a separate container. The larvae were monitored and when maturation was imminent (after 8–10 d), the grown maggots were transferred to 450-ml plastic cups half filled with damp vermiculite. These were maintained at 25°C and 80% RH until eclosion. Sexes of flies eclosing from the different cohorts were then noted. Statistical analysis was by χ^2 test (Zar 1974).

Given the experimental designs, we could not determine if any sexual dimorphism in larval maturation is due to different larval period length or different periods of time spent prior to hatching. To determine if any sexual dimorphism in larval maturation could be explained by sexual differences in egg hatch, the period during which eggs hatch was determined and compared with the time required for a larval cohort to mature and leave the diet. That is, if distribution of eggs hatching over time did not resemble the distribution of larval maturation, then egg hatch differences alone could not account for sexual dimorphism in larval maturation. Eggs were taken from a 24-h collection such as described earlier. Five groups of 100 were held in Petri dishes such as described in the previous section. Neonate larvae were counted and

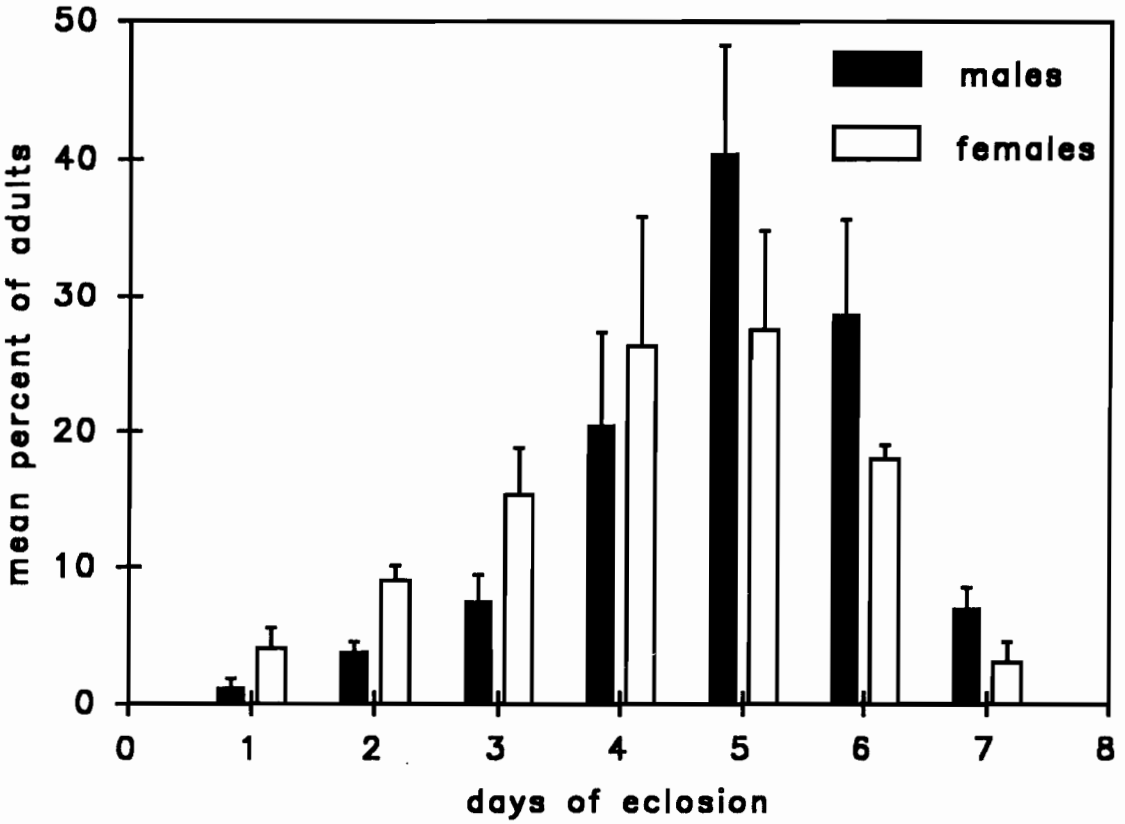

Fig. 1. The mean (+SE) percentage of wild male and female Caribbean fruit flies collected as immatures in fruit eclosing on various consecutive days (n = 4 field collections of 482 pieces of fruit).

removed daily. Statistical comparison of egg hatch distribution and the previously determined larval maturation distribution was by χ^2 test (Zar 1974).

Dimorphism in Sexual Maturation. Earlier eclosion does not necessarily translate into earlier sexual maturity. To determine if males lagged behind females in age of first copulation, individuals of both sexes were given daily sexual opportunities for the first 10 d of adult life. Flies (50 males, 50 females) from a same-age cohort were separately housed in 450-ml plastic cups. A cotton wick protruding from the floor of the cups descended into a lower cup containing water. Food in the form of a brown sugar and yeast mixture (4:1) was placed on a screen in the center of the housing cup lid.

On the day following eclosion (i.e., 1 day of age) each fly was presented with a sexually mature (>10 d old) member of the opposite sex. Matings were recorded, and if no copulation occurred after 1 h, the older fly was discarded. This was repeated on each day until the cohort reached 10 d of age. Statistical analysis was by χ^2 test (Zar 1974).

Results

Sexual Dimorphism in Adult Eclosion. Field samples yielded 1,318 male and 1,639 female flies (45% male). Emergence periods varied from 5 to 8 d in length, and there was a significant difference between the eclosion schedules of the sexes (χ^2 = 49.7, df = 15, $P < 0.005$). Mean female emergence was proportionately greater during the earlier days and mean male emergence greater during the later days (Fig. 1). Subsamples of the four different fruit species revealed that the basic pattern of earlier female emergence was the same in all hosts. In 79 tropical almonds, 67% of the flies eclosing during the first half of the emergence period were female compared with 56% for the latter half. Similar analysis of insects from 122 loquats yielded 100% and 52%; from 52 guavas, 67% and 48%; and from 134 Surinam cherries, 55% and 26%. Field results were confirmed with a large sample of pupae obtained from the laboratory culture in which 463 males and 644 females (42% males) eclosed over 4 d. Again, early emergence was predominately female (χ^2 = 25.1, df = 1, $P < 0.0001$; Fig. 2).

Sexual Dimorphism in Duration of Pupal Period. All of the 140 adults reared from individually isolated larvae eclosed 13 and 14 d after being placed on vermiculite. A significantly greater proportion of females, as compared with males, emerged on the first day (females, day 1 = 61, day 2 = 5; males, day 1 = 39, day 2 = 35; χ^2 = 25.1,

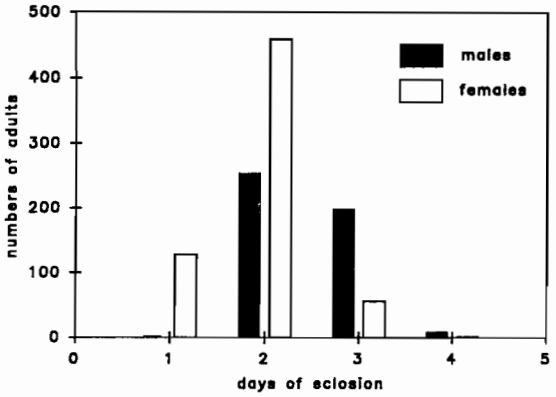

Fig. 2. The number of laboratory-reared male and female Caribbean fruit flies derived from a 24-h egg collection period that eclosed on each of 4 consecutive days.

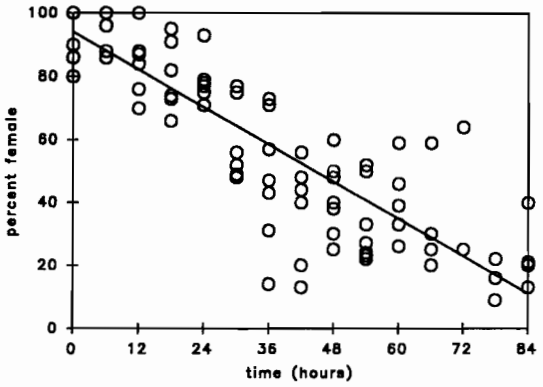

Fig. 3. The percentage of females in samples of mature Carribbean fruit fly larvae taken at consecutive 6-h intervals as they left six diet trays to pupate. Completely overlapping points at a particular sampling point are not shown as distinct.

df = 1, $P < 0.001$), demonstrating that on average, female flies spend less time as pupae.

Sexual Dimorphism in Larval Maturation. Under the conditions described, the first larvae to leave the diet were predominantly female (Fig. 3). During the latter part of the larval collection period, the sex ratio became biased toward males. Time of collection alone explained 73% of the variance in the sex ratios of the samples ($r = 0.85$, $P < 0.0001$). These results were substantiated when samples were taken from days 1 and 3 of larval maturation. A significantly greater number of females was obtained on day 1 (day 1 females, $\bar{x} = 69.2$, SE = 4.8; day 1 males, $\bar{x} = 14.8$, SE = 1.8; day 3 females, $\bar{x} = 16.4$, SE = 3.0; day 3 males, $\bar{x} = 58.2$, SE = 7.5; $t = 11.5$, df = 9, $P < 0.0001$).

Twenty-three percent (SE = 4.4%) of the total number of maggots matured on day 1, 30% (SE = 1.3%) on day 2, 30% (SE = 3.1%) on day 3, and 17% (SE = 2.3%) on day 4. Thus, a substantial number of mostly female larvae should be available by collecting the first day's emergence.

Although male maggots remain in the diet for a longer period of time, they are not heavier than females. Males collected as they left their diet weighed an average 16.5 mg (SE = 2 mg, $n = 70$) compared with 17.2 mg (SE = 2 mg, $n = 69$) for females (male larvae are 96% as heavy as females; $P = 0.03$). However, this sexual dimorphism is not as great as that which occurs in the adult stage. Adult males are approximately 80% the wet weight of females (males, $\bar{x} = 9.7$ mg, SE = 0.3 mg, $n = 44$; females, $\bar{x} = 12.0$ mg, SE = 0.3 mg, $n = 45$; $t = 5.4$, df = 16, $P < 0.001$).

Sexual Dimorphism in Timing of Egg Hatch. Eggs that hatched within 72 h of deposition yielded 86 male and 157 female flies. Those hatching 72–96 h after deposition yielded 68 male and 66 female flies. Larvae that hatched early were significantly more likely to be female than those hatching the following day ($\chi^2 = 7.8$, df = 1, $P < 0.01$). Note that the overall sex ratio was biased toward females (41% male).

Eggs hatched over a 3-d period. Seventy-two hours after collection, 29% (SE = 3%) of the larvae had emerged. Between 72–97 h after collection, 62% (SE = 2%) of the maggots hatched, and between 96–120 h, 9% (SE = 2%) of the maggots hatched. This distribution differs significantly from that of maturing larvae, indicating that sexual dimorphism in hatching rate alone cannot account for the sex ratio pattern among maturing larvae ($\chi^2 = 40.3$, df = 7, $P < 0.01$).

Dimorphism in Sexual Maturation. At the end of 10 d, 47 of 50 males and 39 of 50 females had mated. The mean age of first copulation was significantly earlier for males (6.6 d; SE = 0.19) than for females (7.5 d; SE = 0.21) ($\chi^2 = 37.2$, df = 6, $P < 0.005$; Fig. 4). The earliest age at which any males mated (4 d) also was earlier than the earliest age at which any females mated (5 d; Fig. 4).

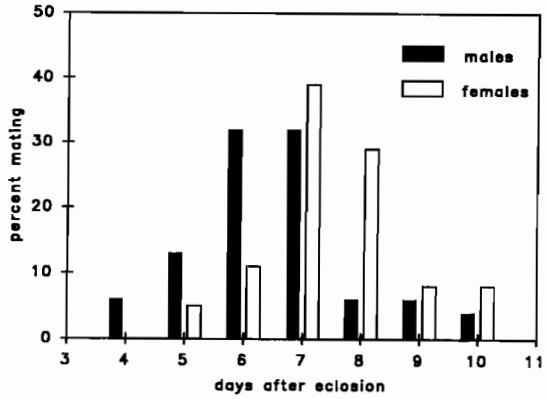

Fig. 4. The proportion of males and females participating in their first copulation on each of the first 10 d after eclosion (n males mating = 47; n females mating = 38).

Discussion

Sexual selection for earlier female emergence is most likely to occur in synchronously eclosing populations with multiply mated females and a high degree of last-male-to-mate sperm precedence (Thornhill & Alcock 1983). Under these circumstances, males might have evolved to avoid the dangers facing adults by delaying emergence, and then concentrate their mating efforts on females just prior to their mate's ovipositing. Female-first eclosion of Caribbean fruit flies is unlikely to be selected in this fashion. First, the earlier sexual maturation of males diminishes the actual difference between the sexes in terms of time between hatching and first copulation. Second, the multivoltine population structure of *A. suspensa* produces only occasional flushes of ovipositing females that follow seasonally peak abundances of host fruit. Such synchronized female egg laying is probably an exception to the rule of what we believe to be more gently fluctuating numbers of setting fruit-oviposition opportunities. Finally, females appear to mate infrequently, unless presented with numerous oviposition opportunities that exhaust sperm supplies (Sivinski & Heath 1988).

It seems more likely that a sexual dimorphism in nutritional needs dictates the differences in time the sexes spend as larvae. Perhaps if females require nutrients that are easily obtained through adult foraging, they may spend less time as larvae and more time as prereproductive adults. It might be argued that rather than females abbreviating larval periods, it is males being selected for extending their time as maggots that accounts for the dimorphism, particularly if males become larger with time and are thus better able to compete for mates (Sivinski & Burk 1989). Note that time spent as a juvenile does not simply translate into size. Female maggots have a shorter development time yet are heavier than males. Still, males might be accumulating resources as larvae, which would allow them to shorten the period of adult foraging and so reach sexual maturity earlier.

Even more puzzling is the apparent propensity of female eggs to hatch before male eggs. It should be noted that sex ratios of wild and laboratory-reared Caribbean fruit flies tend to be female biased, suggesting higher immature male mortality. It is possible that some procedure in our rearing system or experiment inadvertently causes greater male losses among earlier hatchlings.

Caribbean fruit flies are currently being mass reared for projected sterile male releases and are anticipated to serve as hosts for mass reared inundatively released parasites. Exploiting sexual dimorphism in larval maturation may increase the efficiency of such procedures. For example, removing substantial numbers of unwanted females from insects destined for sterilization and release might save storage and transportation funds (these benefits would be greater if dimorphism in egg hatch was exploited). If females were set aside as hosts for parasites, they might directly contribute to biological control efforts. For example, the braconid *Diachasmimorpha longicaudata* (Ashmead) is a candidate for inundative release in Florida, and it is capable of attacking fully mature maggots (although in this case, very mature larvae are not as susceptible as those of a somewhat younger age [Lawrence et al. 1976]). Thus, this biological control agent might be mass reared on early maturing fly larvae containing a disproportionately large number of females.

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